

## STATEMENT OF PROJECT OBJECTIVES

### **Toxicological Evaluation of Realistic Emissions of Source Aerosols (TERESA): Application to Coal-Fired Power Plant-Derived PM<sub>2.5</sub>**

#### **A. OBJECTIVES:**

The overall objective of the TERESA program is to investigate and clarify the impact of the sources and components of fine particulate matter (PM<sub>2.5</sub>) on human health via a set of realistic animal exposure experiments. The DOE-sponsored portion of the TERESA program, covered by a Cooperative Agreement between DOE and EPRI, is designed to assess the toxicity of coal combustion emissions in the Midwestern and Eastern U.S. by exposing laboratory animals to actual plant emissions that have been “aged” and converted to reaction products in a manner that simulates the conversion experienced by coal power plant plumes in the atmosphere en route to ambient receptor sites. Thus, the primary objective of the DOE-EPRI Cooperative Agreement is to evaluate the potential for adverse health effects from ambient exposure to realistic coal-fired power plant emissions. Secondary objectives of the study include: (1) evaluate the relative toxicity of coal combustion emissions and mobile source emissions, their secondary products, and ambient particles; (2) provide insight into the effects of atmospheric conditions on the formation and toxicity of secondary particles from coal combustion and mobile source emissions through the simulation of multiple atmospheric conditions; (3) provide information on the impact of coal type and pollution control technologies on emissions toxicity; and (4) provide insight into toxicological mechanisms of PM-induced effects, particularly as they relate to susceptible subpopulations.

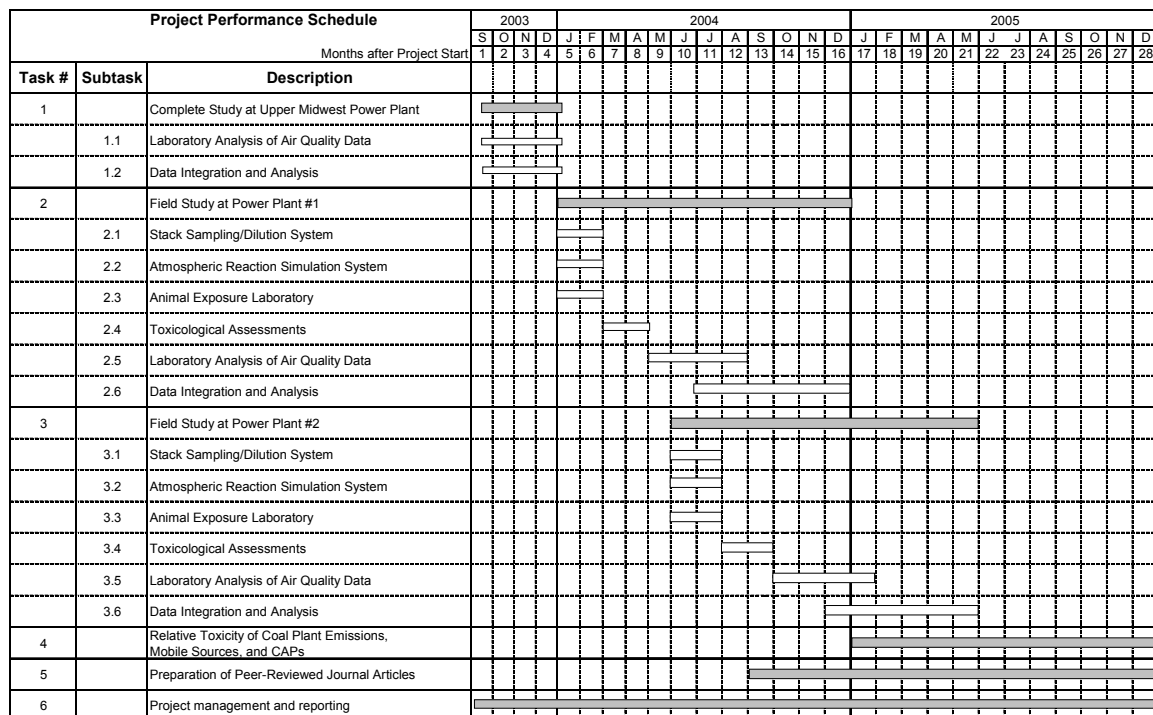
#### **B. SCOPE OF WORK:**

The overall TERESA program, managed by EPRI and including the Harvard University School of Public Health (HSPH) as a key participant, was initiated in July 2002 with non-DOE sources of funding. Prior to the start of DOE-EPRI Cooperative Agreement, it is expected that the following work will already have been completed under the TERESA program: (1) construction of the atmospheric reaction chamber and associated equipment; (2) development and validation of the atmospheric simulation methods; (3) outfitting of the mobile animal exposure laboratories; (4) construction and installation of the custom-built emissions collection/dilution/transmission system at a coal-fired power plant in the Upper Midwest; (4) aging of the primary emissions from the Upper Midwest plant; (5) exposure of normal and compromised rats to emissions from the Upper Midwest plant subjected to different simulated atmospheric conditions; (6) physico-chemical characterization of the various exposure scenario atmospheres at the Upper Midwest plant; and (7) toxicological evaluation of the Upper Midwest scenario atmospheres.

The DOE-EPRI Cooperative Agreement involves the analysis and interpretation of the field data collected at the Upper Midwest plant, followed by the performance and analysis of similar field experiments at two additional coal-fired power plants in the Eastern U.S., utilizing different coal types and with different plant configurations. Since the Upper Midwest plant uses Powder River Basin (Wyoming) coal (with very low sulfur and low ash), it is anticipated that one of the two additional plants will use low sulfur (<1%) eastern bituminous coal, and the other will use medium-to-high sulfur (>2-

In each of the field experiments, stack emissions will be introduced into a reaction chamber, where oxidants will be generated to accelerate secondary particle formation. Laboratory rats will be exposed to the primary and aged emissions and evaluated for pulmonary, systemic, and cardiovascular effects. The DOE-EPRI Cooperative Agreement also includes a comparison of the toxicity of coal power plant emissions, mobile source emissions and concentrated ambient particles (CAPs). Animal exposure experiments to evaluate the toxicity of mobile source emissions and CAPs are also part of the overall TERESA program, but will be performed by the project team independently of the DOE-EPRI Cooperative Agreement. At least 3 manuscripts will be submitted to peer-reviewed journals describing the atmospheric simulations and resulting particle formation, the toxicity of coal combustion emissions, and the comparative toxicity analysis.

For organizational and reporting purposes, the work to be performed under the DOE-EPRI Cooperative Agreement can be broken down into six major tasks: (1) Completion of the initial field study at an Upper Midwest power plant; (2) Field study at power plant #1; (3) Field study at power plant #2; (4) Evaluation of the relative toxicity of coal power plant emissions, mobile source emissions, and CAPs; (5) Preparation of journal articles; and (6) Project management and reporting. The tasks will be performed over a 28-month period according to the schedule shown below, assuming a nominal start date in September 2003.



Each of the six major tasks is described in more detail below.

## **Task 1 - Completion of Initial Field Study at Upper Midwest Power Plant**

### **Task 1.1 - Laboratory Analysis of Air Quality Data**

Many air quality samples will have been collected during the animal exposure experiments conducted at the Upper Midwest power plant prior to the start of the DOE-EPRI Cooperative Agreement. Task 1.1 will include the laboratory analysis of the integrated (filter) air quality samples for particle mass, elements, ammonium, sulfate, nitrate and hydrogen ions, elemental (black) and organic carbon, and particle-associated organic species. Task 1.1 will also include processing and validation of the continuous CO, CO<sub>2</sub>, SO<sub>2</sub>, ozone, NO<sub>x</sub>, particle count, particle size distribution, temperature, and relative humidity data collected during the animal exposure experiments conducted at the Upper Midwest power plant.

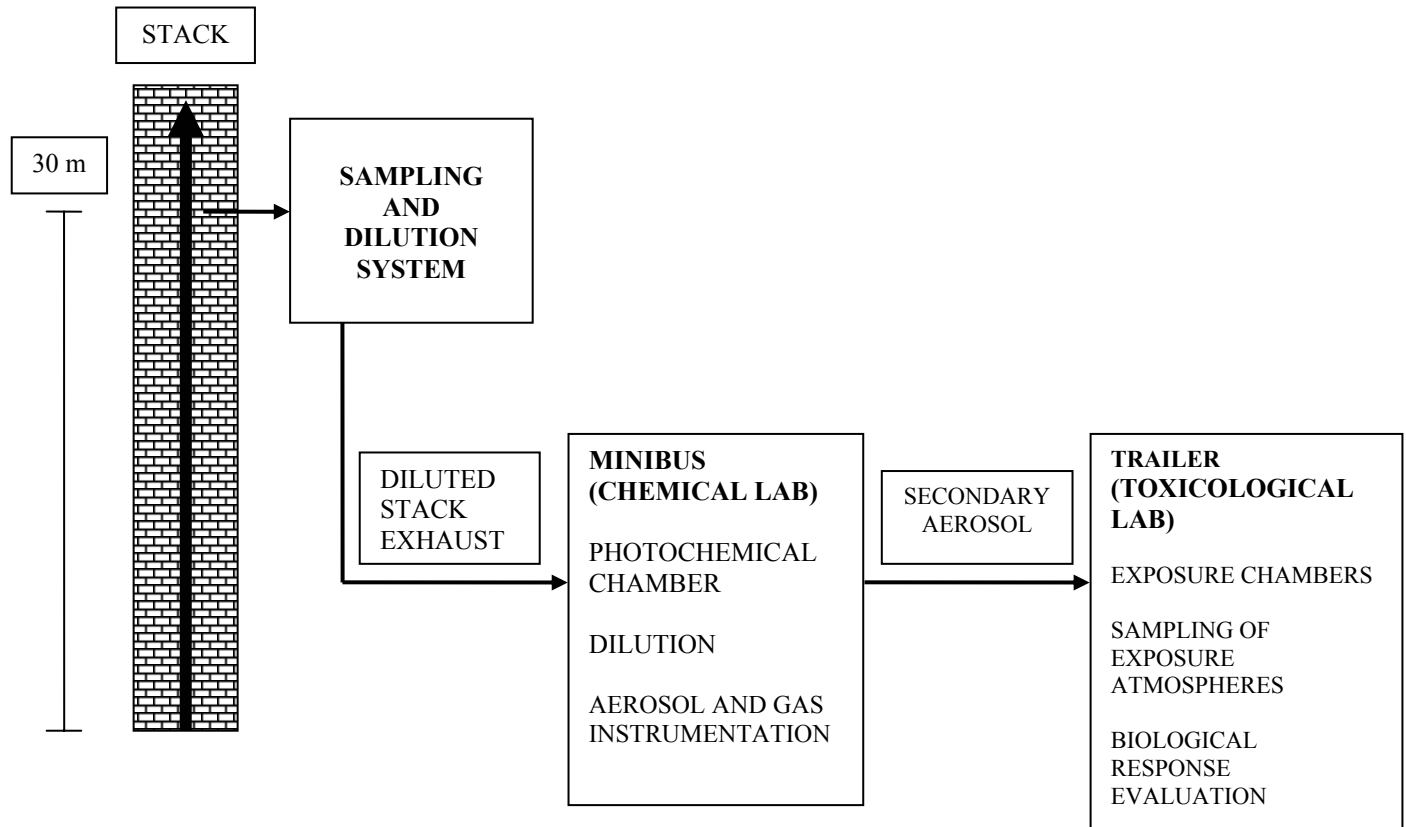
### **Task 1.2 – Integration, Analysis, and Interpretation of Air Quality and Health Effects Data**

A comparison will be made of the biological effects observed during the six exposure scenarios performed at the Upper Midwest power plant - sham, primary emissions, oxidized emissions, neutralized emissions, emissions plus volatile organic compounds, and the gas-phase only (particles removed) for the aged emission scenario which shows the highest health effects (see Task 2.3 for descriptions of these scenarios). For each biological endpoint, two-way analysis of variance (ANOVA) tests will be employed to assess differences. To determine the effect of PM composition on biological response, mixed effects models containing exposure metrics as fixed effects will be fitted to each response outcome measure. Multivariate analyses will be carried out in relationship to various component concentrations. Statistical significance for all analyses will be based on  $\alpha = 0.05$ . An interim report documenting the results of the experiment at the Upper Midwest plant will be prepared. Knowledge gained during the Upper Midwest fieldwork will be used to refine the study design for the field assessments conducted under Tasks 2 and 3.

## **Task 2 – Field Study at Power Plant #1**

As described earlier, Power Plant #1 will use either low sulfur (<1%) or medium-to-high sulfur (>2-3%) eastern bituminous coal. If the plant uses low-sulfur coal, it is expected that it will employ a selective catalytic reduction (SCR) unit (see discussion below in Task 2.3 about possible ammonia generated by the SCR) for NO<sub>x</sub> removal but will not have a post-combustion system for SO<sub>2</sub> removal. If the plant burns medium-to high sulfur coal, it is expected that it will employ a wet or dry scrubber for SO<sub>2</sub> removal; it may or may not use SCR for NO<sub>x</sub> control. A schematic diagram of the entire system to sample/dilute/transport the stack gas, the mobile reaction chamber laboratory, and the mobile animal exposure laboratory is shown in Figure 1.

Figure 1. Schematic of overall system.



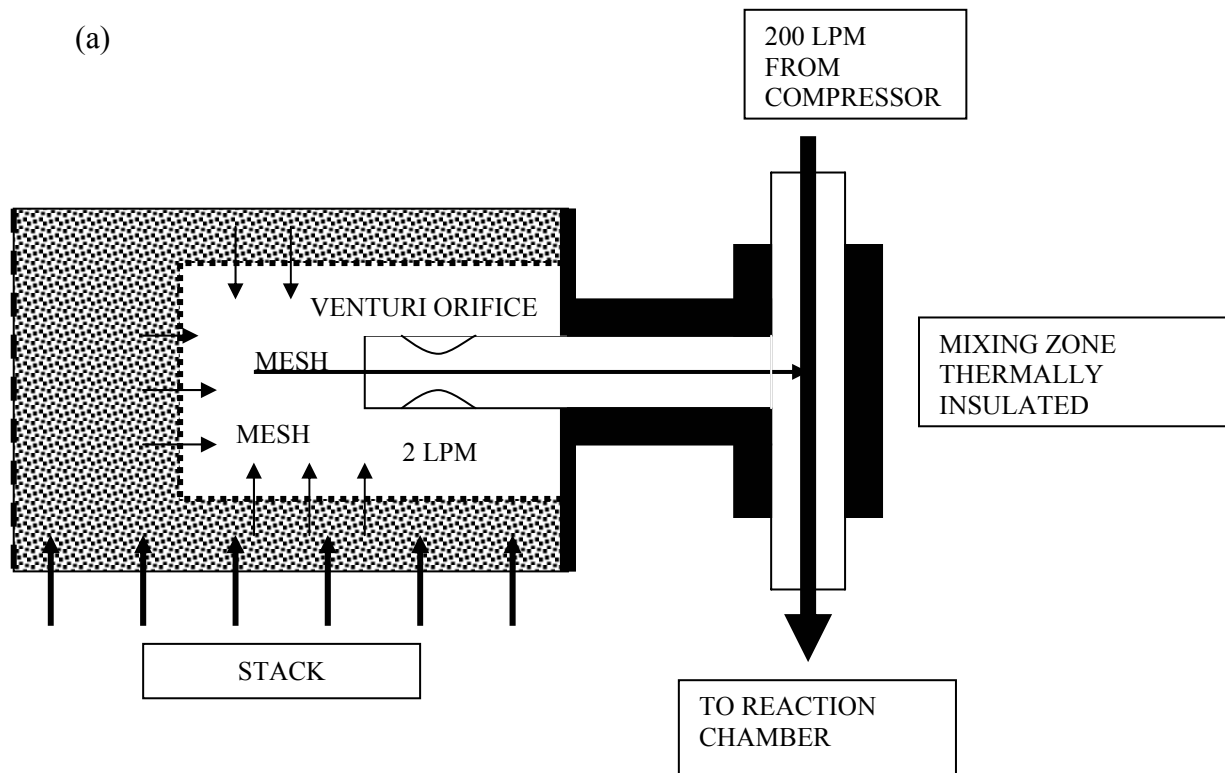
### Task 2.1 – Installation and Operation of Stack Sampling/Dilution System

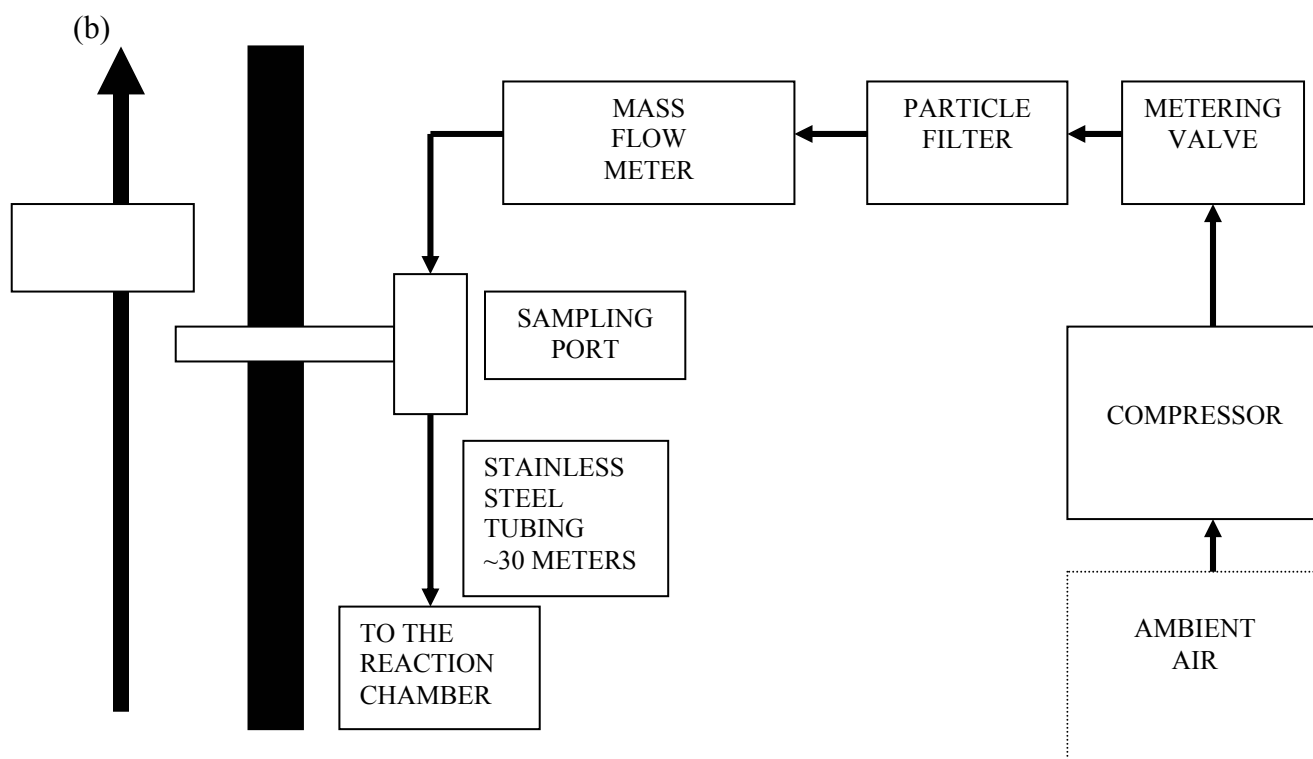
With the assistance of plant personnel, a system (Figure 2) will be installed to collect and dilute emissions from the power plant stack. A stainless steel fine mesh screen will be used to remove particles larger than  $10\mu\text{m}$  order to prevent clogging in the sample flow control and dilution components. A novel design consisting of a novel Venturi critical orifice and a Venturi aspirator will be used to control the flow of dilution air. The Venturi aspirator accelerates a flow of 200 LPM of compressed, particle-free ambient air through a narrow constriction; thus, by the Bernoulli principle, a vacuum is created in a side arm perpendicular to the constriction, which draws the stack gas through the Venturi orifice and simultaneously dilutes it with ambient air. Because the Venturi orifice requires a minimum 10 kPa pressure drop to achieve a sample flow of 2 LPM, a wide range of dilution ratios can be achieved by varying the dilution flow. The dilution air will cool the stack gas to ambient temperature and prevent condensation of water in the sampling line. The diluted stack gas will be transported to the reaction chamber through a 30-meter long stainless steel tube; the relatively high flow of 200 LPM will allow for a very short residence time in the tube, minimizing the losses of ultrafine particles and reactive gases.

Particles larger than 10 microns are removed from the stack gas using a fine mesh screen. Then the stack gas is diluted first during transfer from the stack (factor of 100), and diluted again after the reaction chamber (factor of 10), for a total dilution by a factor of about 1000. It is expected that the mass concentration of both coarse ( $\text{PM}_{2.5-10}$ ) and fine

(PM<sub>0.1-2.5</sub>) particles in the stack gas will be low enough that when this dilution occurs, the contribution of these sizes of primary emission particles will be negligible compared to the secondary fine particles formed from condensation/coagulation in the reaction chamber. Consequently, the most important size range of particles in the stack gas are the ultrafines with size less than 0.1 micron. The ultrafines are also important because they contain trace metals. For these reasons, this is the size range of particles that must be included in the sampled stack gas. These particles will have much higher number concentration, but relatively low mass concentration, compared to the larger particles. However, these ultrafine particles will serve as nuclei for growth of the secondary particles, and are thus the essential component of the stack gas that relates to the ultimate exposure to emission-derived secondary aerosols. All particles will be removed from ambient air used to dilute the stack gas. The pollutant gases in ambient air are several orders of magnitude lower in concentration than in the stack gas, so the concentrations of the gases in the diluted stack gas will be virtually the same as if diluted by pollutant gas-free ambient air.

Figure 2. Sampling and dilution system. (a) sampling port; (b) dilution system.





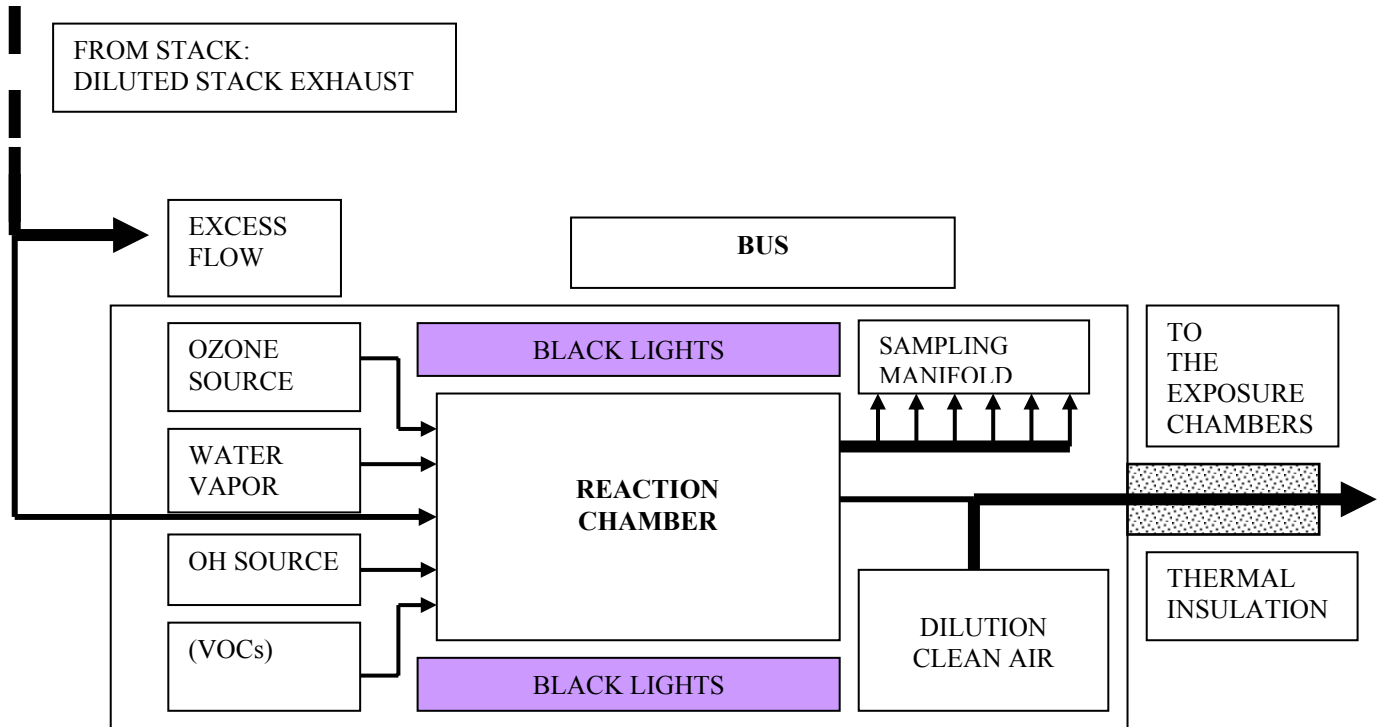
## Task 2.2 – Installation and Operation of Atmospheric Reaction Simulation System

The atmospheric reaction simulation system is a critical component of the TERESA study design, since the basis for the toxicity assessment lies in the generation of realistic exposure atmospheres. The formation, composition, and toxicity of particles will be related to different atmospheric conditions and plume dilution scenarios through variations in reaction conditions.

The atmospheric reaction simulation system will be placed in a mobile chemical laboratory that has already been developed by HSPH through EPRI support and that has recently been utilized in a study in St. Louis. The mobile chemical laboratory is being modified for the purposes of the TERESA study. A schematic representation of the atmospheric reaction simulation system is shown in Figure 3. In the coal power plant setting, diluted stack emissions will flow under positive pressure into a reaction chamber inside the mobile chemical laboratory. In the reaction chamber, diluted stack exhaust is exposed to atmospheric oxidants (i.e., hydroxyl radicals,  $\bullet\text{OH}$ ) to convert  $\text{SO}_2$  and  $\text{NO}_x$  in the stack exhaust to sulfuric acid and nitric acid. The chamber has been designed to oxidize approximately 30% of  $\text{SO}_2$  to sulfuric acid within an approximately 60-minute residence time. Although a larger fraction of the  $\text{SO}_2$  could be oxidized during a longer residence time, it is necessary to minimize residence time in order to (1) keep the chamber small enough for a mobile reaction chamber facility; (2) keep equilibration times sufficiently short to prepare for animal exposures; and (3) reduce losses of ultrafine particles within the chamber. A key reason for maintaining approximately 30%  $\text{SO}_2$  conversion is that it represents a reasonable atmospheric scenario, taking into account transport, deposition, and typical rates of oxidation. By converting a similar fraction in the chamber we will maintain an environmentally relevant ratio of metals to sulfate in the exposure chamber, representative of atmospheres downwind of power plants. Under

typical ambient conditions during warm seasons, SO<sub>2</sub> conversion occurs at a rate of ~3% per hour; to allow reasonable residence times within the reaction chamber, we will increase oxidant concentrations to accelerate aging. The appropriate •OH concentrations will be determined as part of the developmental effort.

Figure 3. Reaction chamber.



The reaction chamber is constructed using 2 mil Teflon film to allow passage of UV light, and measures 12 in x 4 ft x 5 ft (approx 300 L). The chamber configuration was designed to maximize the number of lights while also minimizing the surface-to-volume ratio. The Teflon film has essentially no absorbance in the region of the spectrum of interest (320-500 nm). In addition, Teflon is non-reactive, which minimizes both the potential loss of SO<sub>2</sub> and O<sub>3</sub> on the chamber walls and the formation of secondary products. Photolysis within the reaction chamber is induced using Q-Panel UV 313. The UV 313 lights provide greater light intensity in the lower end of the light spectrum. A filter of cellulose acetate is used to absorb light  $\leq 290$  nm. The OH radical generation system has been already optimized using two prototype photochemical chambers. The most successful system utilizes the photolysis of O<sub>3</sub> induced by the short wavelength light emissions described above. Similar approaches have been used in photobiology for testing biological effects of solar light. The increased energy in the lower end of the solar spectrum should also allow photolysis of carbonyls and dicarbonyls, thereby benefiting the (subsequent) mobile source emissions portion of the study, as well as the scenario utilizing VOCs. Since stack effluent is diluted with ambient air, when necessary, water vapor will be added to the chamber to maintain sufficient humidity (about 60%) to enhance formation of sulfuric acid and particle growth. RH will be monitored continuously and adjusted using a feedback system.

In addition to the diluted stack exhaust and oxidants, other reactants will be added to the

reaction chamber. Some of the exposure scenarios (see Task 2.3) include the addition of ammonia gas (NH<sub>3</sub>) as a partially neutralizing medium for the acidic sulfate aerosol prior to exposure. VOCs (*d*-limonene,  $\alpha$ -pinene or another terpene) will also be added for some of the exposure scenarios to simulate the conversion of VOCs to organic particulate matter from the power plant plume mixing with biogenic emissions. Particulate formation and toxicity from oxidation of these compounds in the presence of ozone has been characterized (Rohr *et al.*, 2002).

To provide the flexibility to proceed with a conversion rate for SO<sub>2</sub> of 30%, a “gas cleaning system” has been designed and evaluated; the system uses a gas-permeable membrane to allow removal of excess SO<sub>2</sub>, NO<sub>x</sub>, ozone, and other pollutant gases, while keeping the secondary particles suspended in air. This system will allow achievement of final exposure atmospheres that have gaseous pollutant levels below concentrations expected to cause health effects, while maintaining particle levels at concentrations at target levels.

The mobile photochemical laboratory will be optimized for fieldwork during the Upper Midwest pilot study (Task 1). Minor changes to compensate for differences of stack emissions and local conditions at power plant #1 are anticipated.

### Task 2.3 – Installation and Operation of Animal Exposure Laboratory

Animal exposures will be performed using both normal and compromised laboratory rats in a temperature- and RH-controlled exposure chamber located in a separate mobile toxicological laboratory (see Figures 4 and 5). Photochemically aged air will be drawn from the atmospheric reaction chamber into a sampling manifold, and diluted with humidity-controlled clean air (ambient air with pollutant gases and particles removed) to maintain the target particle mass levels and to achieve a sulfate particle concentration of approximately 250  $\mu\text{g}/\text{m}^3$ . Air will be drawn through individual exposure chambers in parallel. Exposures will be 4 hours in duration and will be immediately preceded and followed by a 1-hour exposure to humidity adjusted zero air (baseline and recovery periods, respectively). Animals will be maintained and studied in accordance with the National Institutes of Health guidelines for the care and use of animals in research. All protocols will also be approved by the Harvard Medical Area Standing Committee on Animals.

Figure 4. Animal exposure facility.

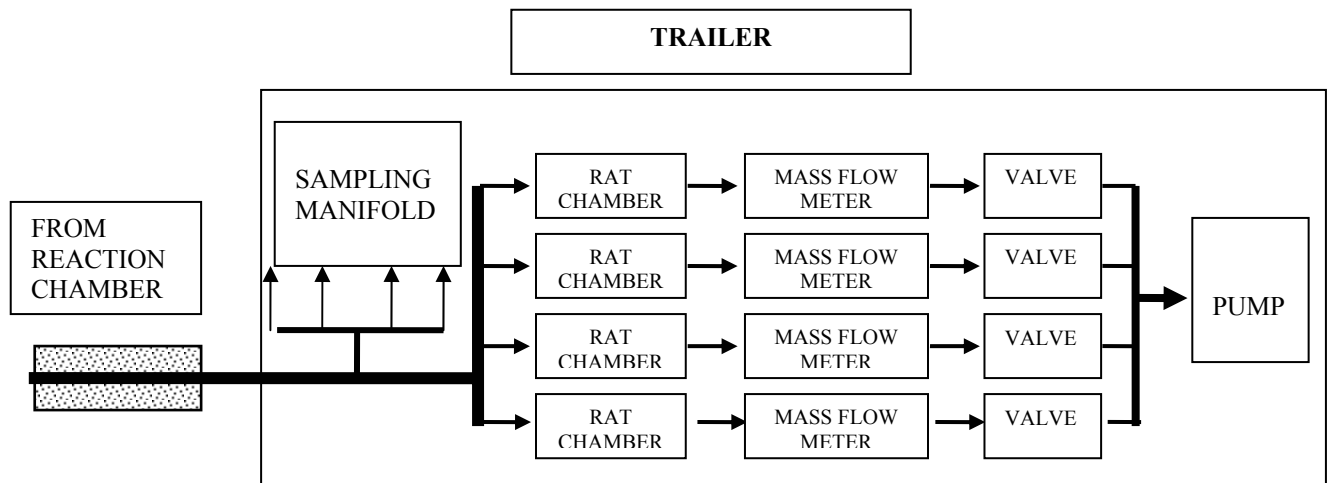
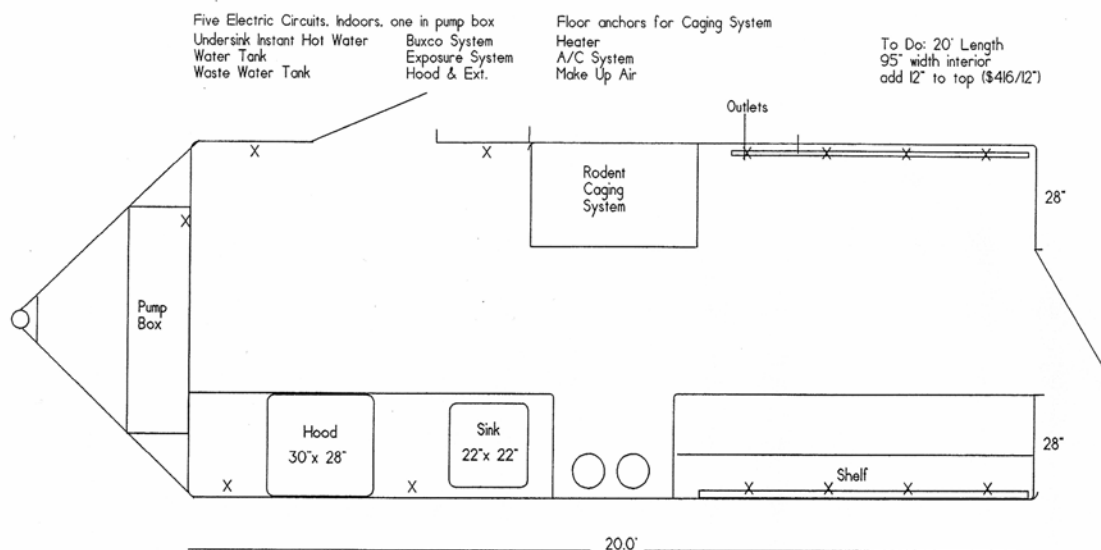




Figure 5. Layout of the mobile animal exposure facility/toxicological laboratory.



The first five of the six different exposures reflect a variety of typical atmospheric conditions as shown in Table 1 below. A sixth control exposure will be conducted using only the atmospheric components (no emissions) of the scenario shown to induce the largest effects of 3, 4, and 5. In addition, laboratory work will be conducted to investigate the contribution of gases versus particles to any biological effects observed by filtering out only particles (not gas phase components) from the atmosphere of the scenario (3, 4, or 5) shown to induce the largest effects.

Table 1. Exposure scenarios and corresponding atmospheric conditions.

Scenario	Composition	Simulated Atmospheric Condition
1	Gas- and particle-free air	Sham exposure
2	Primary (un-aged) emissions diluted to the range of 50 $\mu\text{g}/\text{m}^3$ $\text{SO}_2$ using clean air (same dilution as for 3, 4, and 5 below)	Primary stack emissions
3	Primary emissions + hydroxyl radicals	Aged plume, oxidized stack emissions, sulfate aerosol formation from nucleation
4	Primary emissions + hydroxyl radicals + ammonia	Aged plume, sulfate aerosol partially neutralized by ammonia
5	Primary emissions + hydroxyl radicals + ammonia + VOCs	Aged plume, mixture of neutralized sulfate and secondary organic aerosol derived from biogenic emissions

Exposure atmospheres will be comprehensively monitored for pollutant gases, particle number and size distribution, and inorganic and organic particle composition using an array of continuous and integrated methods. Following transfer from the reaction chamber, the diluted photochemically aged air will be drawn through a manifold that provides sampling ports for characterization, and into an exposure manifold. Sampling will be conducted at four locations: (1) input into the photochemical reaction chamber

(i.e. diluted primary emissions); (2) continuous measurements alternating upstream and downstream of the photochemical chamber; (3) output of the photochemical reaction chamber upstream of the gas cleaning device (i.e. aged emissions); and (4) input into the animal exposure chamber (diluted aged emissions). The locations of the sampling ports are indicated schematically in Figure 6. The specific sampling parameters for each of these locations are described in Table 2 below.

Figure 6. Location of sampling ports.

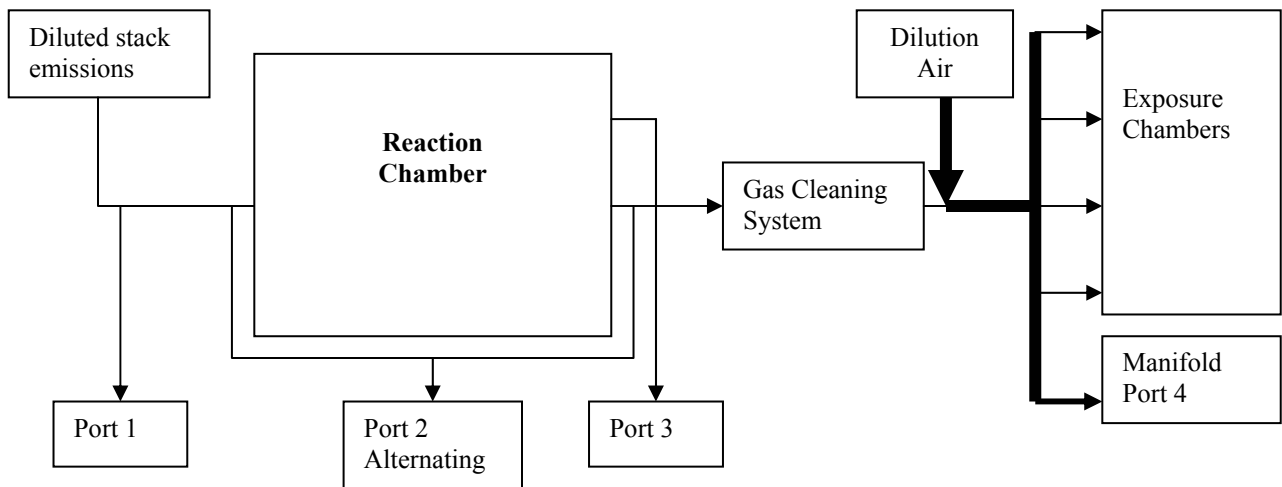


Table 2. Summary of sampling locations and analytical methods.

Site	Process for Measurement	Particles	Gases	Other
1	Chamber Input (Diluted Primary Emissions)	<i>Integrated (TSP)</i> : Mass, $\text{SO}_4^{2-}$ , $\text{H}^+$ , $\text{NO}_3^-$ , EC/OC, $\text{NH}_4^+$ , Specific Organics* <i>Semi Continuous (PIXE Streaker)</i> : elemental analysis		---
2	Chamber Performance (Alternating up and downstream)	<i>Continuous</i> : APS and SMPS (size distribution)	<i>Continuous</i> : $\text{SO}_2$ , CO, NOx, $\text{O}_3$	---
3	Chamber Output (Aged Emissions)	<i>Integrated (TSP)</i> : Mass, $\text{SO}_4^{2-}$ , $\text{H}^+$ , $\text{NO}_3^-$ , EC/OC, $\text{NH}_4^+$ <i>Semi Continuous (PIXE Streaker)</i> : elemental analysis	<i>Integrated (HEADS)</i> : $\text{SO}_2$ , $\text{HNO}_3$ , $\text{HNO}_2$ , $\text{NH}_3$ ; HCHO	<i>Continuous</i> : Temperature, RH
4	Exposure Chamber (Diluted Aged Emissions)	<i>Continuous</i> : TEOM (Mass), CPC (total count), Aethalometer (BC) <i>Integrated</i> : Mass, $\text{SO}_4^{2-}$ , $\text{H}^+$ , $\text{NO}_3^-$ , $\text{NH}_4^+$ , EC/OC,	<i>Continuous</i> : $\text{SO}_2$ , CO, NOx, $\text{O}_3$ <i>Integrated</i> : $\text{NH}_3$ , HCHO	<i>Continuous</i> : Temperature, RH

\*Specific Organics: target combustion derived particulate organics (e.g., PAHs)

Integrated particulate samples for mass, sulfate, nitrate, particle strong acidity, ammonium, and specified organic species will be collected on Teflon membrane filters with no size selective inlets. Since the contribution of the secondary aged aerosol is expected to be much, much greater than the contribution of the primary emission coarse and fine particles (as discussed above), there is no need to separate the coarse or fine particles from the collected particulate samples. Integrated particulate samples for EC/OC analysis will be collected on prefired quartz fiber filters, again with no size selective inlet.

Sulfate, nitrate, and ammonium ion will be measured by ion chromatography, and particle strong acidity will be measured by pH analysis. If power plant #1 uses the SCR, and a significant amount of ammonia is generated, this ammonia will either partially or completely neutralize the strong acidity ( $H^+$ ). It is not feasible to accurately measure the gas phase ammonia concentration. However, measurements of sulfate, ammonium ion, and  $H^+$  will allow us to determine the relative contribution of this ammonia to the composition of the primary emission particles. If there is an excess of ammonia compared to primary emission of acid sulfate particles, then there will be enough to subsequently neutralize part of the secondary acidic sulfate particles produced in the reaction chamber. Thus measurements of both primary and secondary particles will reveal the magnitude of the ammonia effects.

Organic and elemental carbon will be measured by the thermal optical reflectance (TOR) method. Organic speciation of  $PM_{2.5}$  will be conducted by gas chromatography, emphasizing known toxic species of combustion origin (e.g. PAHs). A commercially available circular Streaker sampler will be used to automatically collect sequential particle samples for measurement of both trace metals and black carbon. We expect adequate sensitivity for both measurements using sample durations of about one hour. The collected samples will be analyzed for elements using proton induced x ray emission (PIXE). The filters obtained by the Streaker sampler will also be analyzed by light transmittance, using a custom-built photometer, to determine black carbon.

Size distribution of primary and aged emissions will be evaluated continuously using an aerodynamic particle sizer (APS) and scanning mobility particle sizer (SMPS). Continuous particle count will be measured at the exposure chamber using a condensation particle counter (CPC), and continuous mass concentration will be monitored using a TEOM (Tapered Element Oscillating Microbalance). In addition, continuous black carbon will be measured using an aethalometer.

Continuous measurements of gaseous pollutants will be conducted: CO (non-dispersive IR method),  $CO_2$  (electrochemical sensor method), ozone (UV absorbance method),  $SO_2$  (pulsed fluorescence method), and  $NO_x$  (chemiluminescence method). Gaseous ammonia will be measured by the diffusion denuder technique with ion chromatographic analysis. Formaldehyde will be sampled using DNPH or DNSH coated cartridges and analyzed by HPLC.

#### **Task 2.4 – Performance of Toxicological Assessments**

Normal rats will be exposed to all scenarios and a Stage I toxicological assessment will be performed. The scenario inducing the greatest effects will then be utilized in the Stage

II toxicological assessment using a rat model of myocardial infarction (MI), which is a model of a “heart attack” in humans. Susceptible animal models mimic human diseases or conditions that may make humans more sensitive to the effects of air pollution. These models can help determine which population subgroups are at highest risk as well as provide additional insight into the mechanism(s) of PM effects.

In the Stage I toxicological assessment, pulmonary, cardiac, and systemic effects in normal female Sprague-Dawley rats will be evaluated via bronchoalveolar lavage (BAL), histopathology, pulmonary function, *in vivo* oxidative stress, and blood cytology. Each scenario will include 3 exposures, each with 5 rats (2 for *in vivo* oxidative stress and 3 for the other biological endpoints). Thus, for each scenario there will be 6 rats in the oxidative stress group and 9 rats in which pulmonary function, BAL, and blood cytology are assessed.

Pulmonary function will be evaluated using the BUXCO system (Buxco Biosystem 1.5.3A). Markers of pulmonary function include peak expiratory flow (PEF), tidal volume (TV), respiratory frequency (F), and minute ventilation (MV) (Clarke et al., 1999). Bronchoalveolar lavage (BAL) will be performed, and BAL fluid will be analyzed for cellular content (cell viability, total cell counts, cell type) and biochemical markers of pulmonary injury (lactate dehydrogenase (LDH),  $\beta$ -n-acetyl glucosaminidase ( $\beta$ NAG), and total BAL protein) using standard methodologies. Pulmonary histopathology will be assessed by fixing lungs and randomly selecting three slices for processing by paraffin histology techniques. *In vivo* oxidative stress of heart and lung tissue will be conducted via organ chemiluminescence (CL), a novel method that refers to the ultra-weak light emission produced by biological systems due to the de-excitation of high-energy byproducts of the chain reaction of lipid peroxidation (Boveris and Cadenas, 2000; Boveris et al., 1980). This method has been successfully used in models of oxidative injury in the lung (Gurgueira et al., 2002; Evelson et al., 2000; Turrens et al., 1988; Barnard et al., 1993). Blood cytology (total white blood cell counts and differential profiles) will be evaluated 24 hours following the last day of exposure.

The scenario producing the greatest effects in normal rats (Stage I toxicological assessment) will be repeated using a myocardial infarction (MI) rat model (Wellenius et al., 2002). To produce the MI model, the fine tip electrode of a portable high-temperature thermocautery unit is briefly and repeatedly applied to one or more branches of the left coronary artery. Visible discoloration of the affected region indicates that blood flow has been successfully interrupted. Telemeters for electrocardiogram monitoring will be surgically implanted in Male Sprague-Dawley rats, and monitoring of heart rhythm will be monitored throughout exposure. Blood chemistry and pulmonary function will also be evaluated. For the MI exposures, 2 animals will be exposed at a time. Three exposure scenarios will be assessed: (1) sham (room air); (2) one aged power plant emission scenario; and (3) one aged mobile source emission scenario (see Task 4). Each scenario will be repeated 4 times, for a total sample size of 8 animals in each group.

Cardiac function will be assessed by electrocardiography (ECG), with endpoints of interest including heart rate, heart rate variability (standard deviation of the normal beat-to-beat intervals; SDNN), and arrhythmias. Blood chemistry will be evaluated by measuring complete blood count, circulating cytokines (interleukins-1 and -6), C-reactive protein (CRP), tumor necrosis factor alpha (TNF- $\alpha$ ), and the vasoactive mediator

endothelin-1. All biochemical markers will be determined using standard immunoassay techniques. Pulmonary function will be assessed using the BUXCO method as described earlier.

### **Task 2.5 - Laboratory Analysis of Air Quality Data**

Analogous to Task 1.1, Task 2.4 involves the analysis of air quality samples collected under Task 2.3, including the laboratory analysis of the integrated (filter) air quality samples for particle mass, elements, ammonium, sulfate, nitrate and hydrogen ions, elemental (black) and organic carbon, and particle-associated organic species. Task 2.4 will also include processing and validation of the continuous CO, CO<sub>2</sub>, SO<sub>2</sub>, ozone, NO<sub>x</sub>, particle count, particle size distribution, temperature, and relative humidity data collected during the animal exposure experiments conducted at Plant #1.

### **Task 2.6 – Integration, Analysis, and Interpretation of Air Quality and Health Effects Data**

The biological effects observed during the six exposure scenarios performed at Power Plant #1 (see Task 2.3) will be compared. For each biological endpoint, two-way analysis of variance (ANOVA) tests will be employed to assess differences. To determine the effect of PM composition on biological response, mixed effects models containing exposure metrics as fixed effects will be fitted to each response outcome measure. Multivariate analyses will be carried out in relationship to various component concentrations. Statistical significance for all analyses will be based on  $\alpha = 0.05$ . An interim topical report documenting the results of the experiment at Plant #1 will be prepared.

### **Task 3 - Field Study at Power Plant #2**

Task 3 is completely analogous to Task 2, except that it will be performed at a different coal-fired power plant. If Power Plant #1 used low sulfur (<1%) eastern bituminous coal, Power Plant #2 will use medium-to-high sulfur (>2-3%) eastern bituminous coal, and vice versa. If the Plant #2 uses low-sulfur coal, it is expected that it will employ a selective catalytic reduction (SCR) unit for NO<sub>x</sub> removal but will not have a post-combustion system for SO<sub>2</sub> removal. If the plant burns medium-to high sulfur coal, it is expected that it will employ a wet or dry scrubber for SO<sub>2</sub> removal; it may or may not use SCR for NO<sub>x</sub> control.

Task 3 will involve the same six subtasks as Task 2: (1) Installation and Operation of Stack Sampling/Dilution System; (2) Installation and Operation of Atmospheric Reaction Simulation System; (3) Installation and Operation of Animal Exposure Laboratory; (4) Performance of Toxicological Assessments; (5) Laboratory Analysis of Air Quality Data; and (6) Integration, Analysis, and Interpretation of Air Quality and Health Effects Data. Although each of these subtasks is described under Task 2, it is anticipated that some minor modifications to the experimental procedures may be necessary or advantageous, based on the experience gained from the performance of Task 2.

#### **Task 4 - Evaluation of Relative Toxicity of Coal Plant Emissions, Mobile Source Emissions, and CAPs**

Task 4 involves the integration of air quality and toxicology data collected under Tasks 2 and 3 with similar data generated for mobile source emissions (diesel and/or gasoline engines) and concentrated ambient particles (CAPs). Experimental data for the mobile source and CAPs toxicity assessments will be generated by the Harvard School of Public Health (HSPH) with support from the Harvard/EPA Center for Ambient Particle Health Effects, Grant No. R827353; however, the formal comparative toxicity analyses among the various source types will be conducted as part of the DOE-EPRI Cooperative Agreement.

The mobile source assessment will involve the sampling of diesel and/or gasoline engine emissions directly from a vehicle. The specific type and age of vehicle will be determined through a consultative process with individuals with appropriate expertise. The methodologies for atmospheric simulation, animal exposure, and toxicological assessment will be completely analogous to the methods described under Task 2. The same mobile atmospheric reaction simulation and animal exposure laboratories will be used to ensure similarity of exposure methods and conditions. For the CAPs comparative toxicity assessment, existing CAPs data from the HSPH laboratory will be used.

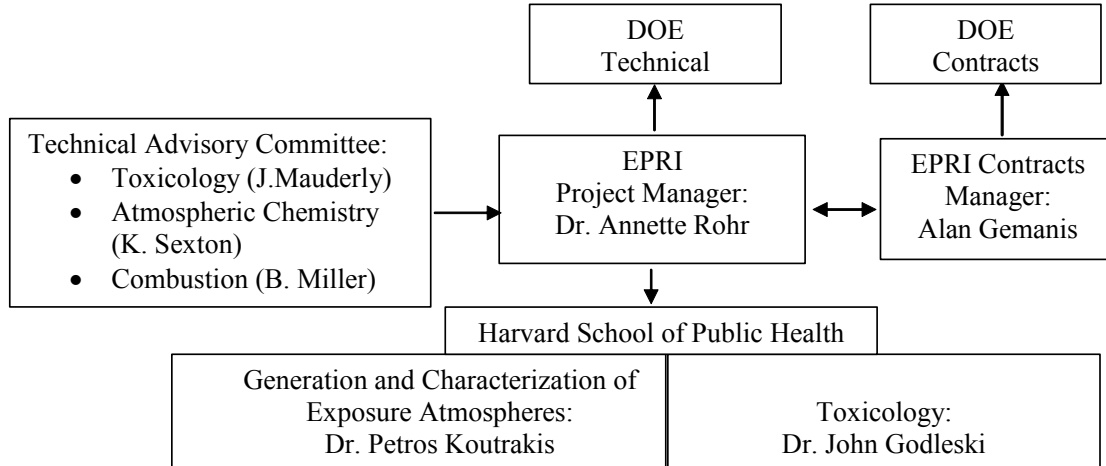
#### **Task 5 - Preparation of Peer-Reviewed Journal Articles**

Preparation of articles for publication in peer-reviewed scientific journals is a critical component of the DOE-EPRI Cooperative Agreement. This task will require a level of analysis and time commitment from project team personnel that goes beyond the level associated with the data analysis and interpretation components of Tasks 1-4 and the project management/reporting functions of Task 6. It is anticipated that articles will be prepared and submitted to peer-reviewed journals on the following three topics: (1) the results of the atmospheric simulation and generation of exposure atmospheres; (2) the results of the coal combustion emissions toxicity assessment; and (3) comparative toxicity assessment for coal combustion emissions, mobile source emissions, and CAPs.

#### **Task 6 - Project Management and Reporting**

Task 6 covers all planning, management, and coordination activities associated with the project. EPRI will coordinate all field, laboratory, data management, and data analysis activities of the subcontractor(s), will arrange appropriate power plant access, and will be responsible for the deliverables/briefings specified in Sections D and E.

The overall Project Manager is Dr. Annette Rohr of EPRI. Dr. Rohr will be supported by Dr. Petros Koutrakis and Dr. John Godleski of the Harvard School of Public Health, as shown in the project organization chart below. Dr. Koutrakis and his team will be responsible for the emissions sampling, exposure characterization, and atmospheric chemistry components of the project. Dr. John Godleski and his team will be responsible for the in vitro and in vivo toxicological assessments. Dr. Rohr will interface with DOE Technical staff to obtain input and feedback on the research program.



A Technical Advisory Committee (TAC) was established to provide input and guidance into the TERESA Program. The TAC is comprised of a toxicologist (Dr. Joe Mauderly, Lovelace Respiratory Research Institute), an atmospheric chemist (Dr. Kenneth Sexton, University of North Carolina), and a combustion engineer (Dr. Bruce Miller, The Pennsylvania State University). The TAC held their first meeting on February 11, 2003 at the Harvard School of Public Health in Boston, MA, and will convene, at a minimum, on a yearly basis through the course of the program.

#### **D. DELIVERABLES**

EPRI will submit the periodic, topical, and final reports in accordance with the Federal Assistance Reporting Checklist contained in the DOE-EPRI Cooperative Agreement. It is expected that semi-annual reporting of technical progress will be performed. For a 28-month performance period, this translates to four semi-annual reports and a comprehensive final report at the conclusion of the project. In addition, EPRI will prepare and submit the three manuscripts (at a minimum) described in Task 5, along with topical reports on the results of the animal exposure experiments at each power plant (Upper Midwest plant, Plant #1, and Plant #2).

It is anticipated that the preliminary results of the project will be presented at a conference or workshop sponsored by DOE, EPRI, or other organizations. Copies of any manuscripts, presentations, etc. developed in support of such conferences/workshops shall be submitted to DOE for inclusion in the project record.

The following is a schedule of key deliverables/milestones associated with the project, based on a nominal start date in September 2003:

Milestone Schedule	2003				2004												2005											
	S O N D				J F M A M				J J A S O N				D J F M A M				J J A S O N				D							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Description																												
Project Kickoff Meeting			▲																									
Complete Study at Upper Midwest Power Plant											▲																	
Complete Field Experiments at Power Plant #1																							▲					
Complete Field Experiments at Power Plant #2																	▲											
Topical Report - Power Plant #1 Results																		▲										
Topical Report - Power Plant #2 Results																											▲	
Semiannual Technical Progress Reports								▲						▲						▲							▲	
Presentations at Technical Conferences										▲												▲						
Manuscripts for Peer-Reviewed Journals																	▲									▲		
Final Report																												▲

## E. BRIEFINGS/TECHNICAL PRESENTATIONS:

A project kickoff meeting will be held within 60 days after project award. It is anticipated that the project kickoff meeting will be held via teleconference because of the diverse geographic locations of the project participants and the travel costs associated with a face-to-face kickoff meeting. EPRI will prepare a presentation summarizing the objectives and work to be performed during the project, and distribute this presentation to all project participants to facilitate discussion during the kickoff meeting.

After each plant site has been selected and before experiments begin, it is expected that meetings/briefings will be held at each host plant site. The purpose of these meetings will be to finalize experimental protocols, clear up any remaining issues regarding plant site support, and answer any questions of the project technical advisory committee. These pre-experiment meetings will be held via teleconference to allow participation by all project technical advisory committee members.

It is anticipated that the preliminary results of the project will be presented at conferences or workshops sponsored by DOE, EPRI, or other organizations. If participation in such conferences/workshops will be supported by the DOE-EPRI Cooperative Agreement, EPRI will provide information (dates, location, etc.) to the DOE COR prior to the event.